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Measurement of depth of burns by laser Doppler perfusion imaging

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Abstract

Laser Doppler perfusion imaging (LDPI), is a further development in laser Doppler flowmetry (LDF). Its advantage is that it enables assessment of microvascular blood flow in a predefined skin area rather than, as for LDF, in one place. In many ways this method seems to be more promising than LDF in the assessment of burn wounds. However, several methodological issues that are inherent in the LDPI technique, and are relevant for the assessment of burn depth, must be clarified. These include the effect of scanning distance, curvature of the tissue, thickness of topical wound dressings, and pathophysiological effects of skin colour, blisters, and wound fluids. Furthermore, we soon realised that to examine the perfusion image generated by LDPI adequately the process of analysis was appreciably improved by the simultaneous use of digital photography. In the present investigation we used both in vitro and in vivo models and also examined burned patients, and found that the listed factors all significantly affected the LDPI output signal. However, if these factors are known to the examiner, most of them can be adjusted for. If the technique is further improved by minimizing such effects and by reducing the practical difficulties of applying it to a burned patient in the burns unit, the technique may find uses in everyday clinical decision-making. © 2001 Elsevier Science Ltd and ISBI. All rights reserved.

Keywords: Burns; Laser Doppler flowmetry; Microcirculation; Skin

1. Introduction

The trend in the treatment of deep second degree and third degree burns is toward very early excision and skin grafting to reduce the risk of infection, decrease hypertrophic scar formation, shorten hospital stay, and reduce costs. Accurate assessment of the depth of a burn at an early stage has therefore become increasingly important in clinical decision-making [1].

Visual and tactile assessments are still the most commonly used techniques in clinically estimating burn depth. They are highly inaccurate, even if done by experienced clinicians [2]. A reliable technique is therefore called for, which ideally should be non-invasive, without risk to the patient, easy to apply clinically, and inexpensive.

Various methods of assessment of burn depth have been suggested, but none has yet achieved widespread clinical acceptance. Injection of radioisotopes [3] and dyes are invasive, as is biopsy. Ultrasound is of value [4], while thermography has been reported to be a useful tool, particularly in hand burns [5,6].

It has often been suggested that the blood flow in injured tissue indicates the extent of tissue damage [7]. Superficial second degree burns have perfusion values greater than those of normal skin, whereas the perfusion in deep second degree and third degree burns is compromised. Microvascular blood flow, and thereby hypothetically the depth of the burn, should be accessible by laser Doppler flowmetry (LDF). Laser light is quasielastically scattered in the tissue, and the back-scattered light is spectrally broadened as a result of moving blood cells. The back-scattered signal is transferred into an electrical signal by a photodiode, and after the signal has been processed the output voltage

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correlates linearly with perfusion [8]. The advantages of LDF over other methods are that it is accurate and non-invasive, although the probe has to be attached to the tissue. The main disadvantage of LDF is that it measures the perfusion in only one single spot.

Several studies have reported the clinical value of LDF in the assessment of the depth of a burn [9–11]. Although the studies are encouraging it must be stressed that like any other technique except visual and tactile assessment, the clinical use of LDF in burn units has not been widely accepted.

A recent development of LDF is laser Doppler perfusion imaging (LDPI), in which a collimated laser beam scans a certain area of the tissue to form an image of its perfusion [12]. Although the theory behind LDF and LDPI is the same, there are several differences. The main advantage of LDPI is that it gives spatial information about the microvascular blood flow. Furthermore, because LDPI is a scanning method, the probe does not come into contact with the tissue.

The use of LDPI in the assessment of the depth of burns in humans was first reported by Niazi et al. (1993) [13], who studied 17 burns in 13 patients. There was complete correlation between the depth of the burn assessed by LDPI and by biopsy. The accuracy of clinical criteria, compared with biopsy, was only 65%. Although this study yielded interesting results, definite proof of the efficacy of LDPI in clinical decision-making has to rely on outcome measures in a prospective, randomised, controlled trial in a large group of patients.

When we applied this technique to assess the depth of burns by measuring tissue perfusion we encountered several methodological issues that needed attention. These issues have instrumental as well as pathophysiological causes including scanning distance, curvature of tissue, and appearance of the wound. One of the most important issues we encountered was how perfusion data could be correlated with the burn wound itself. The perfusion image generated by the laser Doppler imaging system often shows distinct areas with locally altered perfusion values. At an early stage we found it desirable to relate these in the image to the relevant areas in the burn wound. We therefore found digital photography to be an important adjunct for the analysis of the LDPI images that provided additional information.

The aim of the study was to investigate methodological issues in the assessment of the depth of burns by LDPI to obtain an understanding of aspects of the technique that can influence the results. We think that insights into the inherent limitations of LDPI and the practical difficulties of the technique must be systematically addressed when the technique is first applied clinically so that a standard protocol can be developed.

2. Subjects and methods

2.1. Subjects

During a 3-month period outpatients who came into the burn unit were asked to participate in the study. A total of five outpatients (three men) with eight burns gave their consent and were studied. Their mean age was 35 years (range 13–57). Four healthy volunteers (two men) were also studied (mean age 31 years, range 23–38). All were non-smokers and refrained from drinking coffee for 2 h before the experiments. The subjects were sitting comfortably during the measurements. The study was approved by the Ethics Committee, Linköping University Hospital.

2.2. Equipment

A laser Doppler perfusion imager (PIM II scanning system, LDPIwin software version 2.0.9, Lisca Development AB, Linköping, Sweden,) was used to measure blood flow. A laser beam was moved step-by-step through a maximum of 4096 measurement sites by mirrors driven by stepper motors. Perfusion values derived from each measurement site were stored in a computer. A colour-coded perfusion image was generated by the software. Different colours corresponded to different levels of perfusion. Raw perfusion values (volts) were also provided by the software. A DC image, much like a black and white photograph but with a limited resolution, was generated based on the total light intensity back-scattered on to the detector at each measurement site. The tissue surface area contained in the image depended on the distance between the scanner and the tissue.

2.3. Methodological issues

2.3.1. Scanning distance

The effect of scanning distance was investigated in vitro by scanning a fluid containing randomly moving scatterers, covered by a semi-transparent plastic layer 0.1 mm thick. Mean perfusion values were obtained in duplex mode, by averaging over 100 measurement sites. In duplex mode, the scanner continuously measured the mean perfusion of 4×4 sites. Scanning distances were 15, 20, 25, 30, 35, and 40 cm (the typical distances we used in imaging burns).

2.3.2. Curvature of the tissue

In vitro experiments were carried out using a curved semi-transparent surface covering a fluid containing randomly moving scatterers (Fig. 1). The radius of the curvature was 40 mm and scanning distances were 20, 30, and 40 cm. At each distance, a rectangular perfusion image was created that comprised a 180° segment of the curved surface. The incident angle associated with each row of 20 measurement points in the rectangular image was calculated from the position of the row within the image. The incident angle was defined as the angle between the laser beam and the plane normal to the scattering surface. The effect of curvature of the tissue in vivo on the perfusion signal was studied in four healthy volunteers. The forearm was placed in a 42° water bath for 5 min to increase skin perfusion. This also created more homogeneous perfusion. The arm was then dried carefully and an image was made with the scanner placed perpendicular to the flexor aspect of the forearm. The same procedure as in the in vitro experiment was used to obtain the mean perfusion values at different angles.

2.3.3. Dressings and topical agents

To investigate the effect of dressings and ointments used for treatment on the perfusion values, scans were made of the dorsal side of the hands of healthy volunteers ($n = 4$). A baseline measurement was made, followed by a measurement when the skin was covered with either 1) a single layer of silicone dressing (Mepitel®, SCA Molnlycke Ltd, UK), or 2) a 2 mm layer of topical silver sulphadiazine (Flamazine®, Smith & Nephew, UK). The silver sulphadiazine was then wiped off and an additional scan was made. The mean (SEM) perfusion values were calculated from 800 measurement sites (image size: 20×40).

2.4. Laser Doppler perfusion imaging in burned patients

Imaging was done in a dark, temperature-controlled room (22 °C). The patient lay or sat comfortably to prevent movement artefacts. The area around the wound was covered with a dark green cloth to improve the quality of the images. The scanner was placed at a certain distance from the burn, the distance being governed by the surface area of the wound. A maximum scanning distance of 40 cm was allowed. If necessary, markings were made on the healthy skin next to the wound, which showed on the perfusion image as grey, subthreshold areas, because they absorbed most of the laser light. This enabled an accurate match to be made between the image and the digital photograph of the wound (1600-L, Olympus Optical Company, Ltd, U.K.). The images were stored in a personal computer. As the sampling of the LDPI and the digital camera were done manually, the positions of the scanner head and the camera were slightly different. However, this can (to a certain extent) be compensated for during processing of the image. Additional laser Doppler images or digital pictures were taken if necessary.

The images were processed so that the digital image was aligned with the perfusion image. Marked points on the skin indicated areas in the two images that corresponded to each other. Photoshop (Adobe Systems Inc, San José, USA) was used to scale, rotate, and translate the images, as well as to merge different images together. The perfusion image was superimposed on the digital photograph, and made slightly transparent to obtain an image that showed the spatial blood flow distribution within the photograph of the burn in the background. It was also possible to superimpose sequential perfusion images to evaluate changes in blood flow over time.

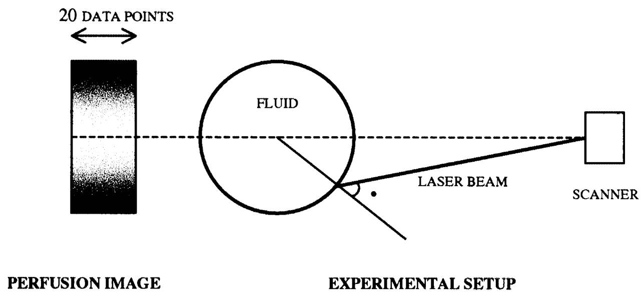

Fig. 1. Experimental design to investigate the effect of tissue curvature in vitro. The laser beam scans the surface with underlying fluid and a rectangular perfusion image is formed. Incident angles are calculated from the position of the measurement site in the image. A similar design is used in vivo, where the fluid is replaced with the subject's forearm.

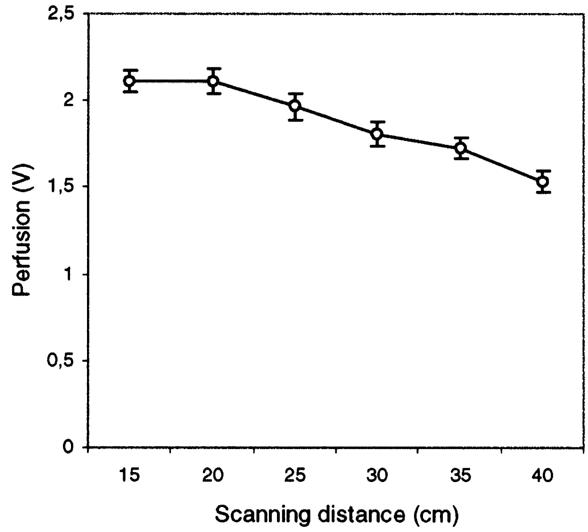

Fig. 2. The effect of scanning distance on the perfusion signal, as measured in vitro.

3. Results

The results of the experiments on scanning distance are presented in Fig. 2. Mean perfusion values decreased with increasing distance; a 27% difference was recorded between 20 cm and 40 cm scanning distances.

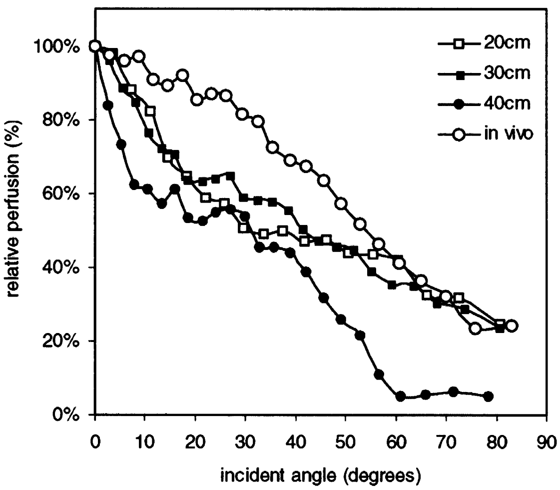

Fig. 3. The effect of tissue curvature on the perfusion image, in vitro and in vivo. Perfusion values are relative with respect to the maximum value at 0° incident angle.

The effect of curvature of the tissue can be seen in Fig. 3. Between incident angles of 30° and 40°, the mean perfusion values decreased to 50% of their maximum at 0° in vitro. In vivo, there was a 50% decrease at an incident angle of 55°. The effects of dressings and topical agents on the perfusion signal are shown in Fig. 4. Both the dressing and the ointment significantly reduced the mean perfusion value. After the ointment had been wiped off the skin there was a slight decrease in the perfusion signal compared with that of normal skin.

A typical result is presented in Fig. 5. It contains the results of two different investigations, done 5 days (a,b,c,d) and 13 days (e,f,g,h) after the burn, respectively. The images are arranged as follows: digital image (a,e); DC image (b,f); perfusion image (c,g); combined image (d,h).

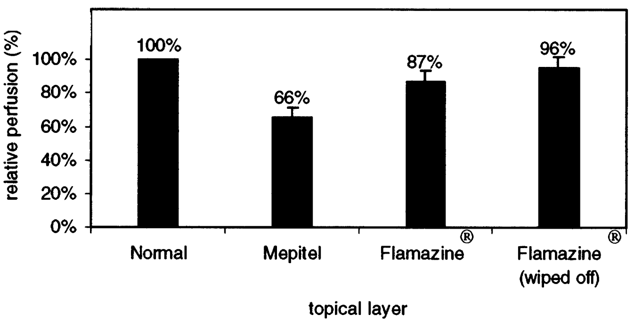

Fig. 4. The effect of Mepitel® and Flamazine® on the perfusion signal. Values are relative with respect to skin without a "topical" layer applied.

4. Discussion

4.1. Laser Doppler perfusion imaging of burns

LDF [9–11] and in particular LDPI [13,14] are valuable in assessing the depth of a burn. However, neither is widely used in clinical decision-making and we think that this is because there are still many questions about the technique that need clarification.

The correlation between perfusion values and depth of burns is not straightforward, as can be seen from our results. A standard protocol must be developed, as well as standards for the laser Doppler techniques. Such a process is already being supported by the European Union [15]. The LDF or LDPI perfusion value is generally expressed in volts. In the assessment of the depth of burns by LDPI, the key question seems to be: what perfusion level corresponds to what depth of burn? It was not the aim of this study to find a definite answer to this question, although as a rule of thumb we found that deep second degree or third degree burns had perfusion levels equal to or less than those of normal skin. Superficial second degree burns had perfusion levels as much as three to five times the level of normal skin.

4.2. Scanning distance

The distance between the scanner and the tissue has been reported to influence the perfusion signal so that perfusion values increased with increasing distance [16]. In this study, on the contrary, we found a decrease in perfusion values at increasing distances (Fig. 2). This discrepancy can be attributed to the use of different imaging equipment. In our system the perfusion signal was normalised to the total light intensity by using the DC signal from the detector in the denominator of the signal processor algorithm. In the system used by Kernick and Shore (2000) [16], however, the square of the DC signal was used for normalisation. The fact that scanning distance influenced the perfusion signal in LDPI implies that care should be taken in interpreting the results of assessments of depth of burns. Although relative values within the same image are not affected by variations in scanning distance, comparison of perfusion data taken from different images can be inaccurate unless the same scanning distance is used for all the images. In the assessment of depth of burns one should be aware that spatial information is lost at longer scanning distances. The scanner in this study used a 2 mm diameter laser beam, and stepping motors making discrete steps of 0.44° to move from one measurement point to the next. This implies that the area of tissue between adjacent measurement points increases quadratically with the scanning distance. However, at scanning distances of less than 25 cm the measurement

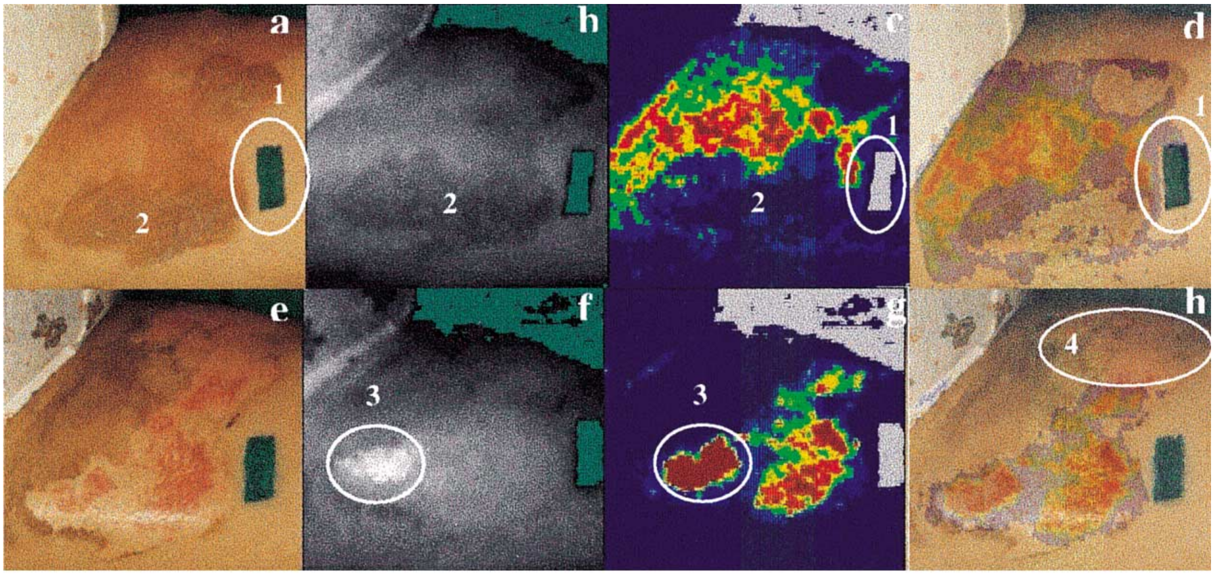

Fig. 5

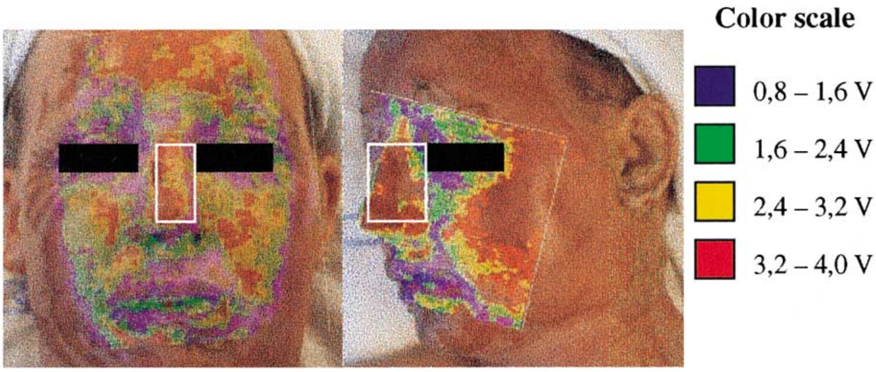

Fig. 6

Fig. 5. Typical result from a LDPI burn depth assessment in this study. From left to right the digital photograph (a,e), the DC image (b,f), the perfusion image (c,g) and the combined digital and perfusion image (d,h) are shown. Area 1 shows the marking made on the skin, which is necessary for alignment of the images. Different imaging artefacts can be recognized: area 2 contains blistering which results in an attenuated perfusion signal that may easily be misinterpreted without the availability of the digital photograph; area 3 shows how direct reflection of the laser beam results in overestimated perfusion values; in area 4 the perfusion values are not included because the tissue curvature resulted in a strong decrease of the perfusion signal.

Fig. 6. Frontal and side view of the perfusion image superimposed onto the digital photograph of a facial burn. Differences in perfusion levels in the marked areas in the two images arise as a result of tissue curvature.

points will overlap because of the finite diameter of the laser beam. We have noticed that scanning distances of over 40 cm resulted in poorer images (results not included).

4.3. Curvature of tissue

Curvature of tissue affects the perfusion signal in vivo. Svedman et al. (1998) [17] found a sharp decrease in valid perfusion data beyond an incident angle of 38°. Valid perfusion values were defined as values that exceeded the instrumental zero level. A different approach in our study led to similar results (Fig. 3). The incident angle of the laser beam strongly affected the perfusion signal in vitro, resulting in a 50% decrease in the range between 30° and 50°. In vivo, the decrease in perfusion values was more gradual as the angle increased, showing a decrease of 50% at an incident angle of 55°. The angle dependency of the perfusion signal may be caused by increased surface reflection and a decrease in backscattered light that reached the detector at larger incident angles. The difference between the in vitro and in vivo findings probably results from the different thickness and optical properties of the semitransparent material and skin. A change in how we normalise the DC may result in a more angle-independent perfusion signal. However, the normalisation in our system could not be controlled. The curvature effect has important consequences for the applicability of the technique in the assessment of depth of burns. Burns are often located over a curved area of the body, particularly those of the extremities and the face. The perfusion values within an image might therefore be reduced as a result of curvature and easily be misinterpreted. An example is presented in Fig. 6. A frontal image and one taken from the side of the face show the same superficial second degree burn. Different colours indicate different ranges in the perfusion level (see legend to Fig. 6). The perfusion image is combined with the digital image to enable accurate mapping of perfusion values on to the burn wound. Perfusion values in some areas of the face, such as the area around the nose and on the cheeks, differ between the two images because of the different angles between the incident laser beam and the surface of the tissue in the different images. In this case, scanning the burn from different directions improves the reliability of the assessment.

4.4. Dressings and topical agents

In the treatment of burns various dressings and topical agents are commonly used to prevent infections, to stimulate the healing process, and to reduce pain. These topical applications lowered the perfusion values (Fig. 4). A silicone dressing such as Mepitel®, although it is thin and transparent, reduced the mean perfusion value by 33%, which might be the result of an increased length of the optical path through the dressing. A layer of silver sulphadiazine ointment in a dose typically used during treatment reduced the mean perfusion value by 13%, presumably because of a relatively large direct surface reflection of the laser light at the surface of the ointment. In both the dressing and the ointment experiments, local cooling by heat transfer from the skin to the topical layer may have lessened the extent of the vasodilation during the measurement. Although one could conclude that these topical layers should not be applied during imaging, relative flow variations are retained within the same perfusion image, and assessment of depth of burns based on blood flow in the wound may still be reliable.

4.5. Pathophysiological aspects

In addition to the scanning height, curvature, and topical layers, several pathophysiological aspects of the burn can influence the perfusion signal. The colour may vary appreciably between burns, and one burn may contain several different colours. Areas within a wound may be dark as a result of necrosis or scar formation, resulting in a high absorption coefficient. This may reduce the perfusion values in these areas, possibly as a result of a reduction in the depth of penetration of the laser light into the tissue. Blisters form a relatively impenetrable layer that may absorb the laser light and reduce the perfusion signal. A wet wound may result in reflection artefacts in the perfusion image.

Fig. 5 shows the digital image, the DC image, the perfusion image, and the digital image with the superimposed perfusion image of a superficial second degree burn on the upper leg. In the digital image (1a), areas with blistering can be recognised. The corresponding perfusion image (1c) shows that the perfusion values in the blistered areas are relatively low. This could easily be interpreted as normal blood flow without the digital image as an extra source of information. In fact, the perfusion signal is reduced by an optically impenetrable layer caused by the blisters, and the actual blood flow distribution below the blistered areas cannot be assessed without removing the blisters.

Another artefact may arise from the direct reflection of the laser beam. The wetness of the wound at the second investigation caused direct reflection in an area clearly seen in the intensity image (1f). The pronounced bright spot indicates an overflow in the signal processor. Although the signal processor normalizes the total light intensity, in areas where the intensity is causing overload of the electronics it does not normalise correctly. This results in overestimated perfusion values, as can be seen in the corresponding perfusion image (1g). There is no argument that the blood flow in the "reflection area" is actually greater than in the other hyperaemic areas.

4.6. Digital photography and sequential imaging

We found that a digital photograph is a valuable, even essential, addition to the perfusion image from a LDPI assessment. The perfusion contains information about variations in spatial blood flow, which are to a certain extent directly related to the depth of the burn. However, several technical or physiological factors may influence the reliability of the perfusion image. Mapping the right perfusion values to the right areas of tissue is also a problem. The use of digital photography results in an accurate match of the perfusion data and the burn itself, and enables visual assessment and assessment by perfusion data at the same time. The quality of the DC image was often insufficient for this purpose.

Fig. 7 shows the value of sequential imaging. Again, the use of digital photography was indispensable, as the superimposed digital and perfusion images enabled accurate understanding of the dynamics in different areas in the burn, in this case during a period of 15 min in which the burn was provoked with heat. There was a

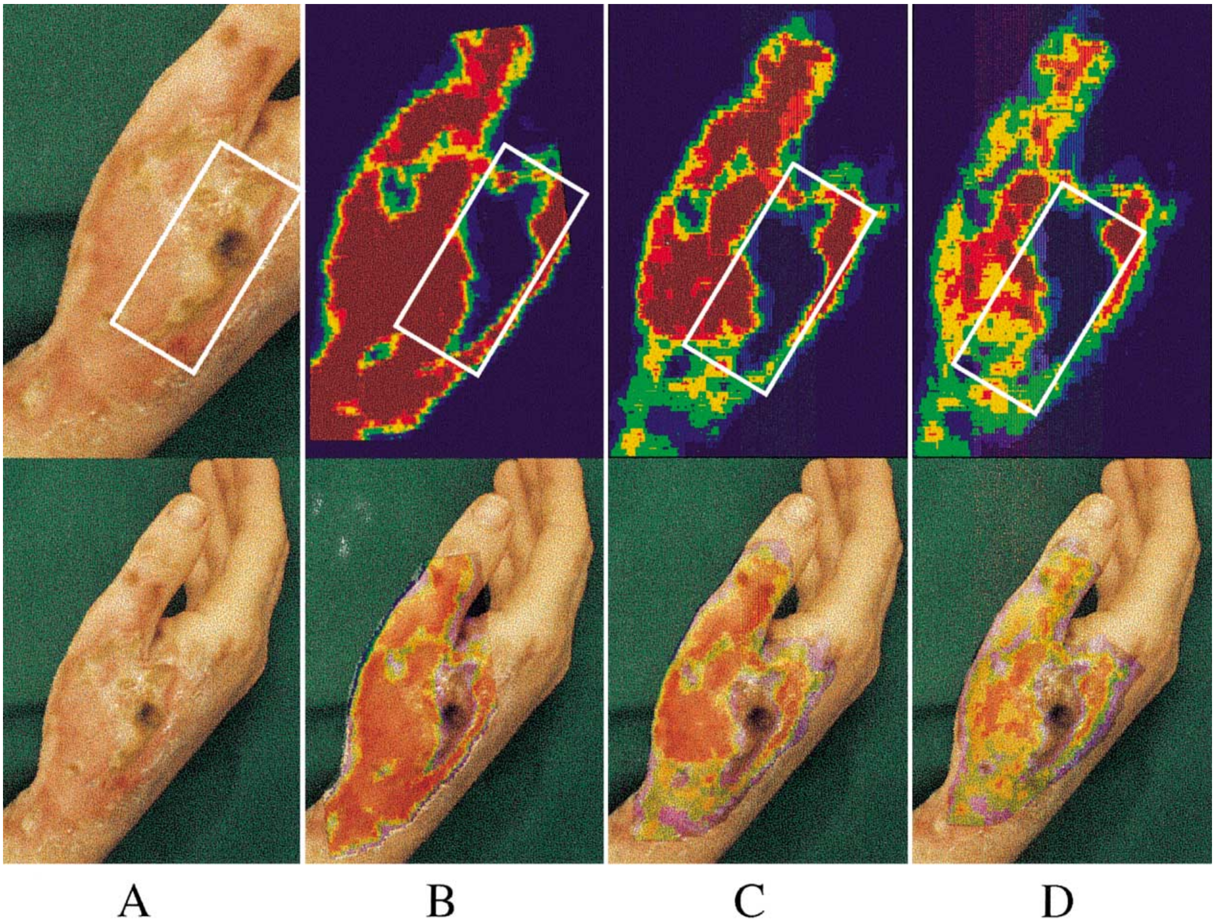

Fig. 7. Sequential images of a hand burn taken during heat provocation with a warm air stream. A: digital images; B: before provocation; C: after 10 min; D: after 13 min. The marked area was a deep burn, excised after 10 days.

decrease in the mean perfusion value as well as local blood flow changes within the burn. An important issue in sequential imaging is the alignment of the images. It may be necessary to apply a marker to the skin next to the burn to align the sequential images, although sometimes the shape of the tissue is sufficient to permit proper alignment. Provocation and subsequent imaging of burns might be a way to gain a better assessment of the depth of burns, as areas of different depths may react differently to provocation. More research is necessary to investigate this.

We conclude that LDPI, together with visual assessment, is a promising investigative tool in the assessment of the depth of a burn. However, a number of instrumental and physiological factors may influence the perfusion signal and may therefore lead to images that are easily misunderstood. Despite this limitation, we think that knowledge of the possible image artefacts, in addition to the availability of a digital photo on which the perfusion image is superimposed, enables a more reliable assessment of the burn wound and therefore improves the value of LDPI.

Acknowledgements

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